POSSIBILITIES OF NITRITE REPLACEMENT IN MEAT PRODUCTS

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Abstract: Ever since ancient times man has sought to increase the sustainability and safety of excess meat and fish. The first methods of conservation were developed based on experience, and later they were developed based on scientific facts. Today, modern production of processed meat can hardly be imagined without the use of preservatives. The most commonly used preservatives in the meat industry are the nitrites: potassium nitrite (E 249) and sodium nitrite (E 250),and the nitrates: sodium nitrate (E 251) and potassium nitrate (E252). Their use limits and prevents the growth and multiplication of microorganisms. At the same time, it also inhibits the growth and multiplication of the pathogenic bacterium Clostridium botulinum. In addition to their preserving effect, nitrites are used in the meat processing industry for the purpose of development and stabilization of the rosy-red color in meat products, prevention of fat oxidation, as well as improving the taste of the finished product. Nitrates, unlike nitrites, do not have a significant impact on the growth and multiplication of microorganisms and the development of other technological benefits. Therefore, they need to be reduced to nitrites with the help of nitrate - reducing bacteria. When nitrites and nitrates are added during the meat processing, nitrite reacts with the amines and amides from the meat. Consequently, carcinogenic and toxic compounds, the N - nitroso compounds are formed. Today, modern consumers are starting to take more care of their health and change their eating habits. They reduce the consumption of processed meat or want to consume naturally processed meat that contains a smaller amount of additives. During the past few decades, much work has been done on the partial or complete replacement of nitrites with alternative natural sources of nitrites in the production of processed meat.
Keywords: nitrites, nitrates, substitute, antimicrobial effect, safety

1. INTRODUCTION

Meat is a product which spoils very easily. It is an excellent medium for the growth of microorganisms due to several factors: the high moisture content, the presence of nutrients, the nitrogen compounds, and the favorable pH (about 5.6). Therefore, it is necessary to prevent the spoilage of meat caused by microbial activity as soon as possible.

Nitrites and nitrates are additives belonging to the group of preservatives. They prevent intoxication and spoilage of meat and meat products, i.e., extend their shelf life (Kovačević et al., 2016).

Shelf life increasing and safety of meat products, as well as food in general, marks the mankind development through history. At the beginning, the preserving methods have been developed on experience only, but later the development have been based on scientific facts. Nowadays, modern food production is hard to be imagined without preservatives, among which the most commonly present in meat industry are nitrites: potassium nitrite (E 249) and sodium nitrite (E 250) and nitrates: sodium nitrate (E 251) and potassium nitrate (E 252) (Silovska and Belichovska, 2020a).

Nitrites are one of the additives that are currently widely used in the production of meat products along with salt, sugar, ascorbate, and polyphosphates (Goswami et al., 2014). They are mainly used to maintain the safety and quality of products (flavour, color), and to prevent lipid oxidation (Danev, 1999; Alahakoon et al., 2015).

Nitrates, unlike nitrites, have no antimicrobial activity, but with the action of the genus Micrococcus, they are reduced to nitrites with the help of the enzyme nitrate-reductase and thus serve as a source of nitrites, the concentration of which is significantly reduced during processing. Thus, the antimicrobial effect is also reduced, which is further contributed by the higher pH value, the reducing effect of the ascorbic acid, the thermal treatment, i.e., the long-term maturation and storage (Tompkin, 2005).

The use of nitrites in combination with other factors contributes to the reduction and prevention of the growth and multiplication of pathogenic bacteria, the most famous of which is *Clostridium botulinum* (Pavlinić Prokurica et al., 2010; Srebranek and Bacus 2007 a, b; Sindelar and Milkowski 2011; Kovačević et al., 2016, Lee et al., 2018), whose toxin (botulinum) causes botulism (Hotchiss and Cassens 1987), which is one of the deadliest foodborne illness. Botulism was a serious health problem before nitrites were used as preservatives in meat products (Archer, 2002).

Pradhan et al., 2009 state that nitrites and nitrates inhibit the growth and multiplication of other pathogens such as *Salmonella spp., Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* and *Bacillus cereus*.
Nitrites also have an inhibitory effect on bacteria of the genera: *Achromobacter, Aerobacter, Escherichia, Flavobacterium, Micrococcus* and *Pseudomonas*, while *Sallmonellae* and *Lactobacilli* are resistant to nitrites (Person and Smooth, cited by Pejkovski, 2000).
The effect of nitrites also applies to gram-negative bacteria such as *Escerichia coli*. However, their effect on *Micrococcus, Enterococcus* and *Lactobacilli* is smaller (Weber, 2004).
Tompkin (2005) found that nitrites are effective in controlling gram-negative enteric pathogens, such as *Salmonella* and *Escherichia coli*. Pichner et al., (2006) indicate that, in fermented sausages, nitrites have an inhibitory activity on *Enterobacteria* and the *shiga toxin- producing Escherichia coli (STEC)*.
Nitrites have long been known to have antimicrobial effect.They prevent the secretion of toxins produced by the following bacteria: *Clostridum botulinum, Straphlococcus aureus* and *Yersinia enterocolitica*, which can develop in the aerobic environment in vacuum packaging. (Archer, 2002).
The nitrites in meat products inhibit the growth of foodborne pathogens and bacteria that spoil food by a variety of mechanisms, including oxygen uptake and disruption of oxidative phosphorylation, nitric acid production (HNO3), and nitrogen dioxide (NO2) breakdown of critical enzymes in bacterial metabolism, such as aldolase (Weiss et al., 2010). Nitrates are used to prevent and control the growth of *Clostridium botulinum* and other pathogens that are commonly found in meat products. As the nitrite concentration increases, so does the growth inhibition and secretion of the toxin *Clostridium botulinum*. Huhtanen and Wasserman (cit. Lee et al., 2018).
In meat products, nitrates act synergistically with sodium lactate and sodium diacetate against *Listeria monocytogens* (Seman et al., 2002; Gill and Holley, 2003; Lagan et al., 2004).
Ha et al. (2016) indicate that reduced nitrite concentrations in meat products may result in a large increase in food spoilage bacteria (*Lactobacillus spp., Enterococcus spp.*, and *Pseudomonas spp.*) and the development of pathogenic microorganisms (*Listeria monmonytogenes, Staphylococcus aureus*) in meat products.
The added additives in the meat processing are in accordance with the legislation. The most widely used and most common additives in the meat products are the nitrites. However, we cannot deny the fact that the use of nitrites has an adverse effect on the consumers' health (Silovska and Belichovska, 2020b).

2. POSSIBILITIES FOR REPLACEMENT OF NITRITES IN MEAT PRODUCTS FOR THE PURPOSE OF MICROBIOLOGICAL SAFETY

Nitrites react with secondary amines during the production, heat treatment or storage process to produce nitrosamines. (Pavlinić Prokurica et al., 2010; De Mey et al., 2017). Numerous studies have been conducted in the past forty years in order to reduce the use of nitrites, and thus reduce the risk of the formation of harmful carcinogenic N-nitroso compounds in meat products.
According to Raseta et al., (1975),the removal of nitrites in cured meat products is meaningless until a suitable replacement is found. To solve this problem in the meat processing industry, it is necessary to look for an appropriate way to mitigate the negative effects resulting from the use of nitrites.
Wirth (1991) worked on cured meat products and found that for the development of typical color and aroma of cured meat, it is necessary to add 30-50 ppm nitrite (depending on the type of product), while to inhibit harmful pathogenic microorganisms (such as *Clostridium botulinum, Salmonella spp.* and *Staphylococcus spp*.), 80-150 ppm nitrite is required.
The problem of eliminating nitrites from meat products and replacing them with other innocuous additives is complex. All efforts have been unsuccessful to find an equivalent nitrite substitute. Many of the tested alternative additives can replace only one of the effects of nitrites, and only with a combination of additives is it possible to provide all the effects of nitrites (Wirth, 1991). Also,O'Boyle et al., 1991; Shahidi and Pegg (1992) point out that it is very unlikely to find a single compound that will replace all the functions of nitrites.
In 1975, the United States began producing a mixture of nitrite-free curing additives. This curing alternative contained the antimicrobial agent methylparaben, tert-butylhydroquinone.
The study conducted by Eskandari et al., (2013) aimed to replace nitrites with the use of dye, antioxidants and antimicrobials. Koshinel (carmine E120) was used as a colorant, butylated hydroxyanisole (BHA) was used as an antioxidant, and sodium hypophosphite (SHP) was used as a botulism antitoxin and antimicrobial agent. Sodium hypophosphite (NaPO2H2) was used as an antimicrobial agent up to 1000 ppm in products containing low levels of nitrites, i.e. 3000 ppm in meat products in which no nitrites were added. It also inhibits the production of Clostridium botulinum at 25 ° C for at least three days (Eskandari et al., 2013).
Lactates are used in the meat industry because they slow down the development of undesirable microflora in meat and meat products. They can be used as a substitute for nitrites. The action of lactates is based on the bacteriological

action of their ability to reduce water activity. The effect of lactates on preventing the growth of microorganisms in the products is explained by the lactate effect. In an undissociated environment, lactate diffuses into the cells of microorganisms where it dissociates and contributes to an increase in oxygen. (Paul et al., 2007).

Veber (2004) points out that lactate ion has a bacteriostatic effect. His study states that many microorganisms can be inhibited by lactates. These include *Staphylococcus aureus, Listeria monocytogenes, Clostridium botulinum, Campylobacter, Salmonella, Brochotrix termosphacta, Yersinia enterocolitica*. Sodium lactate (60%) added in an amount of 3.3% has the effect of delaying the growth of *Listeria monocytogenes* for several weeks (Choi and Chin, 2003).

Sebranek and Bacus (2007 a, b) point out the possibility for production of the so-called natural or organic meat products without direct addition of nitrates and nitrites. They indicate the natural substitutes of nitrates and nitrites: tartaric acid, lemon juice, cherry powder, celery powder, rosemary and others. The shelf life of these processed meats was significantly shorter than the shelf life of the processed meat in which nitrites were added.

Mediterranean sea salt can also be used as a natural source of nitrites and nitrates because it contains 1.1 ppm nitrates and 1.2 ppm nitrite. Generally, sea salt is obtained by evaporation of seawater, unrefined, without additives, and serves as a natural source of minerals. Solar evaporated sea salt must contain at least 97.5% sodium chloride and, to a certain extent, calcium, magnesium, arsenic and heavy metals (Sebranek and Bacus 2007 a, b).

Gassara et al., (2016) point out that spices such as: nutmeg, oregano, sage, black and white pepper, garlic, ginger, cinnamon, cloves, and others have bactericidal and fungicidal effect. They limit the growth and multiplication of microorganisms that cause food spoilage. Also, these spices stimulate the production of lactic acid (after the action of bacteria of the genus *Lactobacillus*), which affect the extension of shelf life in meat products.

Garlic is added to meat products, primarily due to its antimicrobial effect. Freshly squeezed garlic juice prevents the growth of gram-negative bacteria. Garlic powder, garlic oil and allicin have shown to have antimicrobial effect against *Staphylococcus aureus, Salmonella typhi and Listeria monocytogenes* (Dankert et al., 1979). Reducing the number of nitrites in meat products can be achieved with the use of garlic.

In addition, it is necessary to ensure proper storage of meat products, while keeping their flavour and aroma. If used in large quantities, garlic can significantly change the flavour and aroma of the finished product. For these reasons, it is necessary to minimize the amount of garlic added. Gassara et al., (2016) indicate that garlic, in combination with cloves, inhibits the growth of pathogenic bacteria.

Clove oil shows an inhibitory effect on *Listeria monocytogenes*. Gassara et al., (2016) point out that mixtures of cinnamon and clove oil are used as an alternative to chemicals used in packaging and control, as well as to suppress the mold and yeast growth.

Rosemary oil has antibacterial (Domokos, 1997) and antifungal properties (Daferera, 2000). Rosemary extract has an antioxidant activity due to diterpene phenols and flavonoids that act as hydrogen donors and free radical scavengers (Aeschbach and Prior, 1996).

Thyme gives a strong aromatic flavour to meat products. Thyme extracts inhibit the uptake of fats into meat as a result of the antioxidant action of rosemary acid, essential oil and flavonoids (Haraguchi, 1996). Extracts and essential oils also have a pronounced antimicrobial effect (Hansel 1999).

Oregano essential oil has a pronounced anti-enterobacterial effect (Aureli, 1992). Flavonoids are present in the form of glycosides of luteolin, apigenin and naringenin (Antonescu, 1982).

The use of starter cultures in industrial production increases the hygienic safety of food, its nutritional value, improves its sensory characteristics, equalizes and improves the quality of the product, increases the durability of the product and production is more profitable (2000; Incze, 2002; Incze, 2003; Martinović and Vesković Moračanin, 2006).

Today, modern requirements for a high level of safe and quality food impose complete control over the production process. Therefore, the scientific focus of research is on the potential use of starter cultures. Previous research has yielded a great number of positive results in preventing the proliferation of potential pathogens and improving sensory characteristics (Maksimović et al., 2015; Laranjo et al., 2017; Silva et al., 2018).

Martinović and Vesković Moračanin (2006) list the major metabolites produced by lactic acid bacteria. In the production of fermented sausages, they list: organic acids (lactic, formic, acetic, etc.), hydrogen peroxide, diacetyl, carbon dioxide, bacteriocins, etc. They point out that some of these metabolites, although having an adverse effect on the maturation process, which affects the quality of the product, are still of great importance as inhibitors of the development of unwanted microflora. Most of the metabolites act effectively on the microorganisms: *Pseudomonas spp., Clostridium tyrobutyricum, Brochothrix thermosphacta* which cause spoilage of the finished product, and they also control the growth of enterobacteriaceae *Escherichia coli, Yersinia enterocolitica, Listeria monocytogenes, Clostridium perfringens* (Laranjo et al.2019).

Nitrates and nitrites are naturally present in some vegetables. Beets, broccoli, celery, cabbage, lettuce, radish, and spinach contain high concentration of nitrates (more than1000 mg/kg), whereas, when it comes to fresh vegetables, nitrites are present in very low concentrations (less than 1 mg / kg) (Gassara et al., 2016). Vegetable juice and powder contain a much higher concentration of nitrates compared to fresh vegetables (Eisinaite, 2016).

The reduction of nitrate to nitrite takes place under the action of reducing, i.e. denitrifying bacteria. Bacteria from the genus *Micrococcus*, species *Staphylococcus xylosus, Staphylococcus carnosus, Kocuria* (formed by *Micrococcus*) (Sebranek and Bacus, 2007.b; Bassi et al. 2015) can be distinguished as reducing bacteria. Reduction of nitrates to nitrites can occur by the action of naturally occurring microflora in meat or by added bacteria (starter cultures) due to the activity of the enzyme nitrate reductase (Sebranek and Bacus, 2007.a .; Hammes 2012).

The alternative natural sources of nitrates present in high concentrations in some vegetables, and the use of starter cultures, can contribute to minimizing or eliminating the use of nitrates. Madentzidou et al., (2012) found that in the production of traditional Greek sausage with fresh leeks mixed with salt, the presence of lactic acid bacteria in the filling is significantly increased and thus causes a faster decrease in the pH value of the filling. In sausages, microbial activity and stability are improved, production time is shortened and sensory characteristics are improved. Eisinaite et al., (2016) point out that adding 3% lyophilized celery, parsley and leek in dry fermented sausages has no significant effect on changing the pH, the water activity, the lactic acid bacteria, coagulase - positive staphylococci present during the process of fermentation and ripening of dry fermented sausages compared to control sausages, in which there are no incorporated vegetables. They point out that the best sensory characteristics have been observed in sausages that have lyophilized celery juice added.

3. CONCLUSION

The production of meat products that do not contain or contain very little nitrite has been the subject of much research by researchers for more than four decades. No compound can yet be found that can completely replace all the positive technological effects of nitrites in the production of processed meats. The use of natural nitrites present in large quantities in some vegetables, in combination with starter cultures, as well as the modern technology, can contribute to the production of meat products without the use of a synthetic source of nitrites. However, it is still necessary to work on this topic in order to confirm the safety of these products because food safety is one of the key factors for consumer health.

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